RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 1, 5-8, 10-14, 17-22, 26, 27, 31-33, 35-41, 43-45, 49-52, and 54 were pending. Claims 1, 14, and 31 have been amended to more clearly claim the Applicants' invention. Claim 1 has been amended to recite the limitations of claim 6. Support for the amendment of claims 1 and 14 is found, for instance, in claim 6 as filed, and in the Specification, for instance at page 4, lines 22-26. Claims 5-6 are cancelled without prejudice, in view of the amendment of claim 1. Support for amendment of claim 31 is found for instance at page 7, lines 5-10. Claim 8 is amended to correct a typographic error. Claims 1, 8, 11, 12, 14, 18, 20, 31, 36-39, 44, and 50 have been amended as requested by the Action. No new matter has been added. Thus, claims 1, 7, 8, 10-14, 17-22, 26-27, 31-33, 35-41, 43-45, 49-52, and 54 are presented herein for reconsideration.

B. Claim Objections

The Action has objected to claims 1, 8, 11, 12, 14, 18, 20, 28, 31, 36-39, 44, and 50 because it is stated that the term "media" should be in singular form. Applicants note that "media" is commonly used in either the singular or plural form. However, to advance prosecution, and because it does not change the scope of the claims, the claims have been amended to recite "medium" in place of "media". Withdrawal of the objection is respectfully requested.

C. Claims rejections under 35 U.S.C. § 102(b)

Claim 1 remains rejected under 35 U.S.C. § 102(b) as being anticipated by Smith *et al.* (*In Vitro* 13:329-334, 1977), in that Smith *et al.* are alleged to disclose a method of culturing cotton callus under dark conditions. Applicants respectfully traverse.

Applicants note that conditions for callus formation and for embryogenesis/plant regeneration are distinct, employing, for instance, different levels of plant growth hormones. Thus it is surprising that Smith achieved any plant regeneration (*i.e.* from callus cells) even from cotyledon tissue. Since this "regeneration" event was unique and not repeatable, Applicants respectfully submit that the "cotyledon" piece, described as having "regenerated" a plant, likely (but inadvertently) contained a node comprising an apical meristem, allowing for such "regeneration" to occur even under growth conditions designed for callus proliferation.

Further, the Action seems to assert, at page 4, first paragraph, that it would have been obvious to subject promising callus, proliferating under the conditions described by Smith, to embryogenesis. This appears unclear, first since the remark is made in the context of an anticipation rejection, rather than an obviousness rejection. Additionally, Applicants again note that conditions for callus formation and for embryogenesis are distinct. While cotton callus formation has been known for many years, perhaps since the early or mid 1970's (*e.g.* cited Davis and Smith references), conditions leading to successful cotton embryogenesis, embryo maturation, and plant regeneration were not defined until much later. Indeed, the present application is precisely concerned with optimizing conditions for cotton embryogenesis, embryo maturation, and plant regeneration. Thus, methods leading to formation of only cotton callus tissue do not anticipate the presently claimed invention.

Regarding the allegation that callus proliferation occurred under dark conditions, Applicants also respectfully traverse. The Action continues to assert that Smith describes such growth. Applicants respectfully disagree. Smith, at page 333, states that high light and low light conditions were preferable to dark conditions, however, in making this statement Smith does not state anywhere that callus proliferation occurred under dark conditions. The assertion that there was

callus proliferation under dark conditions is a misinterpretation of the reference. Applicants submit that any rejection made on this basis is in error, and respectfully request that it be withdrawn.

Finally, Applicants note that claim 1 has been amended to recite hypocotyl tissue. To anticipate a claim, the cited reference must teach each and every element as set forth in the claim. MPEP 2131. Smith *et al.* do not describe any embryogenesis or regeneration of cotton callus tissue derived from a hypocotyl. On the contrary, although hypocotyl tissue was described as a tissue source for *G. arboreum* callus formation, no embryogenesis or regeneration was observed from this tissue (*e.g.* Smith, page 332, last paragraph, right column, and following). As the cited reference does not recite all of the limitations of claim 1, it does not anticipate the claim. Applicants thus respectfully request that the rejection be withdrawn.

D. Rejection of Claims 1, 5-6, 8, 10-12, 14, 17, and 18 under 35 U.S.C. § 103(a).

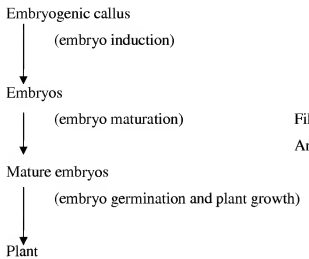
(1) Rejection of Claims 1, and 5-6

The Action rejects claims 1, 5-6, 8, 10-12, 14, 17, and 18 under 35 U.S.C. § 103(a) as being unpatentable over Firoozabady *et al.*, in view of Davis *et al.* and further in view of Chi *et al.* Applicants respectfully traverse the rejection of claim 1, while noting that claims 5-6 have been canceled. Applicants note that claims 1, 8, and 14 are independent claims, and that the rejection of claim 1 is apparently made on the basis of the Firoozabady reference. As the above rejected claims comprise 3 independent claims, Applicants disagree with the assertion in the Action, at page 4, 5th full paragraph, that the claims are, as a group, drawn to a method of culturing cotton cells in medium containing antioxidant and ethylene inhibitor, in the dark. Applicants point out that each of these limitations may be utilized separately, and traverse the rejections separately.

Applicants note that the Action concedes that the Firoozabady 1993 reference does not describe any result of embryogenic cotton callus culture in dark conditions (*e.g.* Action, page 4,

bottom). Further, although the Action asserts that embryogenic callus was cultured in media under complete darkness, referring to the cited reference, at page 169, right column, last paragraph, this paragraph does not describe such culture conditions for embryogenic callus. Instead, the callus referred to as grown in darkness at page 169 is non-embryogenic callus, growth of which having just been initiated and is being maintained, in the absence of embryogenesis. This is clearly the case because (1) the callus of the second and third sentences of this paragraph is not referred to as embryogenic callus; and (2) the following (fourth) sentence bridging pages 169-170 explicitly relates to embryogenic callus formation, further indicating the distinction between (non-embryogenic) callus and embryogenic callus. Applicants also note that the cited reference teaches away from using darkness for embryogenic callus formation and growth, instead teaching that "high temperature and low light ($9\mu E/ m^{-2} s^{-1}$) were preferred..." [page 170, left column; underlining added]. Although the Action points to page 171, right column 1st full paragraph, of Firoozabady regarding growth of embryos to produce plants, Applicants respectfully note that this portion of the cited reference does not describe any lighting conditions for such growth. To clarify, a schematic flowchart showing some of the steps is provided as follows:

Growth Stage	invention	Firoozabady 1993
Explant (*e.g.* hypocotyl piece)		
↓ (callus initiation)		light does not matter high temp
Callus		
↓ (embryogenic callus formation)	Dark antioxidant sealing	low light high temp



The addition of Davis *et al* (*In Vitro* 9:395-398, 1974) or of Chi *et al.*(*Pl. Cell Rep.* 9:195-198, 1990) is not asserted to be for the purpose of curing any defect in the rejection of claim 1; instead these references are explicitly stated to be combined with Firoozabady for the purpose of the ascorbic acid and AVG limitations of independent claims 8 and 14. These references do not cure the defect in the rejection of claim 1, regarding growth of cotton callus tissue in the dark to obtain embryogenic callus. The cited references therefore neither teach nor suggest all elements of the claims to one of skill in the art, and a *prima facie* case of obviousness has not been established. In view of this, withdrawal of the rejection of claim 1 under 35 U.S.C. §103(a) is respectfully requested.

(2) Rejection of claims 8, and 10-12 under 35 U.S.C. § 103(a).

The Action combines Firoozabady (1993) with Davis to reject claims 8, and 10-12, relating to use of an antioxidant such as ascorbic acid (Action, page 5, 5th full paragraph). However, Applicants again note that the cited Davis reference does not relate to growth of regenerable or embryogenic callus, or to culturing callus in an embryo-inducing medium. Indeed the terms "embryogenic" or "embryogenesis" are not even found in Davis. As noted previously, the term "regeneration" is found at page 397, left column, 3rd line. However this term is used in the context

of undifferentiated callus maintenance and proliferation, in view of removal of portions of tissue from a culture being maintained for callus growth. The term does not refer to regeneration of a plant by inducing formation of an embryo.

At page 5, 5th full paragraph, the Action asserts that Davis was combined to show that ascorbic acid might be added to enhance the growth of callus tissue [underlining added]. Applicants again note that claims 8, and 10-12 do not relate to growth of callus tissue *per se* but rather to growth of embryogenic callus tissue in an embryo-inducing medium. One of skill in the art of cotton cell culture would understand that a cotton callus cell culture is distinct from a cotton embryogenic callus cell culture, for instance in terms of the conditions under which it is grown and the physiological state and developmental potential of the cells.

The art of cotton cell culture at the time of the Davis reference had not successfully grown embryogenic cotton cells, or cotton cells that were regenerable. The Davis reference, to the extent that it even might apply to culture of embryogenic cotton cells, which Applicants do not concede, would provide no expectation of success, since induction of embryo formation is known to require different conditions than maintenance of a non-differentiated callus culture. In view of the above, the cited references therefore neither teach nor suggest all elements of the claims to one of skill in the art, and a *prima facie* case of obviousness has not been established. Applicants respectfully request that the rejection of claims 8, and 10-12 under 35 U.S.C. §103(a) be withdrawn.

(3) Rejection of claims 14, 17, and 18 under 35 U.S.C. § 103(a).

The Action combines Chi *et al.* with Firoozabady to support the rejection of claims 14, 17, and 18 (see Action, page 6, 1st paragraph). Applicants respectfully traverse.

Applicants initially note again that *Brassica* species are not closely related to cotton plant species. A brief comparison of some of the differences between *Brassica* and cotton cell culture and regeneration is provided:

Cotton	**Brassica**
Perennial	annual
Woody	herbaceous
Family Malvaceae	Family Cruciferae
Regeneration via embryogenesis (as described)	Regeneration via organogenesis
$AgNO_3$ does not promote regeneration	$AgNO_3$ reported to promote regeneration
Hormones used in cell culture: 2,4-D and kinetin	Hormones used in cell culture: BA and NAA
regeneration time: ~20 weeks	regeneration time: ~10-14 days

Among the many differences described above, importantly, regeneration in Brassica is via organogenesis, which is developmentally distinct from the embryogenic regeneration of cotton described in the present application. That is, in Brassica, formation of embryos is not required to yield the structures that eventually grow into plants. Instead, organs (*i.e.* shoots) can form directly from dividing cells within explants. In view of the limitations of claim 14, which specifically relate to formation of embryogenic cotton callus, the teachings of Chi simply do not apply to the claimed invention, and would give a skilled practitioner no expectation of success in improving the efficiency of cotton plant regeneration. Applicants respectfully submit that the rejection is based on an oversimplification of the cited reference, and respectfully request that it be withdrawn.

Applicants also note that Chi *et al.* state that "$AgNO_3$ was generally more effective in promoting shoot formation than AVG, which appears to be inhibitory to shoot regeneration of ssp. chinensis and ssp. parachinensis..." [Chi, page 196, last paragraph, and following to page 197; and

Table 1]. Later (page 197), the reference states "The effect of AVG and $AgNO_3$ on shoot regeneration varies with genotype and explant source". Thus, again, if there were that much variation in response to AVG among subspecies within a single species (*B. campestris*), one of skill in the art would simply have had no expectation of success in routinely applying the teachings Chi *et al.* to an entirely different family of plants. The Action provides no rationale as to how application of Chi *et al.* would be applied to Firoozabady in view of this. For this reason as well, a *prima* facie case of obviousness has not been established, and Applicants respectfully request that the rejection of claims 14, 17, and 18 under 35 U.S.C. §103(a) be withdrawn.

E. Rejection of Claims 7, 13, 19-22, 26-27, 45, and 49 under 35 U.S.C. § 103(a).

The Action rejects claims 7, 13, 19-22, 26-27, 45, and 49 over Firoozabady *et al.* (1993), in view of Davis *et al.* and further in view of Chi *et al.* and further in view of Gould (*Plant Cell Rep.* 10:12-16, 1991) under 35 U.S.C. § 103(a). The rejection apparently relates to the limitations that recite that the claimed tissue is transformed or transgenic. Applicants respectfully traverse.

Applicants submit that the Gould reference describes transformation of cotton apical meristems. Thus, it essentially represents an alternative approach whereby transformed cotton plants may be obtained without the necessity of taking cells through somatic embryogenesis. Thus, the reference is not apt, as it does not apply to the claimed invention. A brief summary of some of the differences between these approaches for obtaining transformed cotton tissue is supplied as follows:

Embryogenesis	Apical meristem approach
Explant contains no preformed buds	explant: contains preformed buds
Explant tissue: hypocotyl or cotyledon	Explant tissue: shoot meristem
Uses a callus stage	does not use a callus stage
Produces embryos in culture	does not produce embryos in culture
Regenerates from a single cell	regenerates from a group of organized cells

Hormones used: 2,4-D and kinetin	Hormones used: IAA and kinetin
Regeneration via embryogenesis	Regeneration via organogenesis
Genotype dependent	Genotype independent
Resulting plants have "extensive phenotype abnormalities"	Resulting plants "exhibited normal phenotype"

(Quotes are from Gould *et al.*)

The Gould reference contains repeated comparisons of their described technique with that of cotton regeneration via embryogenesis, highlighting numerous differences. For instance, the presently claimed methods require callus formation, in order to subsequently produce embryogenic callus, embryos, and plants. In contrast, at page 14, left column, 2nd paragraph, Gould discusses using low levels of IAA, in order to maintain apex organization and to avoid callus formation.

Further, Gould's description of the chromosomal and phenotypic abnormalities seen when cotton plants are regenerated via somatic embryogenesis as compared to their described apical meristem approach teaches away from use of an embryogenesis-based system. This and the numerous other differences between the somatic embryogenesis and shoot apical meristem approaches described above also clearly indicate that a skilled practitioner would have no expectation of success in applying the teachings of Gould to the methods of the present invention.

Indeed, it is entirely unclear which of the teachings of Gould might be applied to the methods of the present invention in order to achieve any successful result at all, and Applicants respectfully request such teachings be pointed out. One of skill in the art of cotton cell culture and transformation simply would not apply the teachings of Firoozabady, Davis, and/or Chi, relating to callus cell culture of cotton and somatic embryogenesis of cotton, with the teachings of Gould, which relate to culture and transformation of cotton apical meristems and regeneration of plants via

organogenesis. No *prima* facie case of obviousness has been established, and the Applicants thus respectfully request that the rejection be withdrawn.

F. Rejection of Claims 31-33 and 35 under 35 U.S.C. § 103(a).

The Action alleges that claims 31-33 and 35 are unpatentable under 35 U.S.C. § 103(a) over Firoozabady *et al.* (1993) in view of Davis *et al.* and further in view of Chi *et al.* and further in view of Firoozabady (1987), in that cotton tissue is stated to be cultured on a support matrix such as filter paper.

Applicants note in response that Firoozabady (1987) only describes use of filter paper for transformation, during co-culture of cotyledon pieces with *Agrobacterium*. This is an early step in the overall transformation and regeneration process, and the use of filter paper is described as being in order to avoid overgrowth of bacteria on plant tissues. Embryogenic cotton tissue is not being placed on filter paper; rather, cotyledon pieces are being placed on filter paper. After the co-cultivation step, Firoozabady (1987) teaches that plant tissues be transferred to growth medium without filter paper (page 107, right column, 3rd full paragraph). This teaches away from use of filter paper during steps subsequent to co-cultivation. In contrast, claims 31-33 and 35 explicitly recite that the use of filter paper is in conjunction with transgenic embryogenic cotton tissue, following induction of embryogenesis of already transformed but non-embryogenic tissues. The use of filter paper therefore is recited to be well after any co-cultivation or other transformation step.

Applicants also note that the step in Firoozabady (1987) that would be most comparable with the presently claimed use of filter paper is actually found at page 108, first paragraph left column, section entitled "Regeneration of transgenic plants". In this section, Firoozabady (1987) describe induction of embryogenesis in previously transformed (but non-embryogenic) callus, to produce and germinate somatic embryos. No use of filter paper at this stage is taught or

contemplated by Firoozabady (1987). Thus it is unclear to Applicants how the teachings of Firoozabady (1987) would render these claims obvious, even if they were applied, and no *prima facie* case of obviousness has been established. However claim 31 has been amended to clarify the step in the process in which the filter paper is used. In view of the above, withdrawal of the rejection is respectfully requested.

G. Rejection of Claims 36-38 and 35 under 35 U.S.C. § 103(a).

The Action rejects claims 36-38 as being unpatentable over Gould *et al.* in view of Rangan (U.S. Patent 5,244,802), under 35 U.S.C. § 103(a). Applicants respectfully traverse.

As noted above, the teachings of Gould relate to culture of cotton shoot apical meristems, in which plants are regenerated via organogenesis. No callus step is required, or desired, and as also noted above, Gould explicitly attempts to avoid callus formation, which would interfere with the organization of the apical meristem tissues and their ability to produce a regenerated plant via organogenesis. In contrast, as noted by the Action, Rangan relates to cotton plant regeneration in which casein hydrolysate is added to a medium designed to promote embryogenesis during callus cell growth.

Although the Action states (page 10, 2nd full paragraph) that " (i)t would have been obvious…to use the method of culturing transgenic embryogenic cotton tissue as taught by Gould…," Applicants respectfully note that Gould does not teach or describe culturing of embryogenic cotton tissue. Instead, Gould describes apical meristem culture, and repeatedly distinguishes between these two culture approaches for cotton plant regeneration. The Action also asserts that a skilled practitioner "…would have been motivated to combine the methods of Gould with the method of Rangan because casein hydrolysate may further develop the somatic embryos into plantlets…" It is entirely unclear to the Applicants how addition of casein hydrolysate to an

apical meristem culture taught by Gould would be applicable, let alone that one of skill in the art would have any expectation of success in doing so, or even any motivation to attempt such a combination. Gould does not describe or contemplate use of somatic embryos in any way, instead repeatedly teaching away from the somatic embryogenesis approach for cotton plant regeneration. The two references relate to distinct approaches for regeneration of cotton plants. No *prima* facie case for obviousness has been established. Withdrawal of the rejection is respectfully requested.

H. Rejection of Claims 39-41, 43 and 44 under 35 U.S.C. § 103(a).

Claims 39-41, 43, and 44 are rejected under 35 U.S.C. § 103(a) as unpatentable over Firoozabady (1993) in view of Davis *et al.,* in view of Chi *et al.,* in view of Firoozabady *et al.* (1987), and further in view of Rangan. Applicants respectfully traverse.

Applicants first note that claims 39-41, 43, and 44 are drawn to the culture of regenerable non-embryogenic cotton callus tissue, and not to culturing non-regenerable cotton callus tissue, as asserted in the Action (page 11, top). Claim 39 explicitly recites that non-embryogenic tissue is cultured via use of an antioxidant, an ethylene inhibitor, and dark lighting conditions to produce embryogenic tissue, and use of a support matrix, and an amino acid hydrolysate is then recited in culturing the derived embryogenic tissue. As noted above, Firoozabady (1993), Davis, Chi, and Firoozabady (1987), taken together, do not teach or motivate a skilled practitioner to practice the claimed invention, such as by use a support matrix (*e.g.* filter paper) in culturing embryogenic tissue. Firoozabady (1987) teaches away from such an attempt, since filter paper is only utilized with cotyledon pieces (non-embryogenic, non-callus tissue), and filter paper is taught to be removed prior to induction of embryogenesis. The addition of Rangan does not cure this defect regarding use of a support matrix. Rangan only describes use of filter paper for insect bioassays (Examples 14-15).

Further, Rangan is asserted to be combined in view of its teachings regarding addition of amino acid hydrolysate. Although Rangan describes use of amino acid hydrolysate with non-transformed tissue (embryogenic callus and embryos), during what is apparently an embryo maturation and germination phase of growth, this does not relate to the limitations regarding prior culture of non-embryogenic cotton callus tissue under dark conditions, as recited in the claims, and hence Rangan does not cure the defect of Firoozabady (1993) in the rejection of these claims.

As noted above (*e.g.* section D (1)), Applicants note that the Action concedes that the Firoozabady 1993 reference does not describe any result of embryogenic cotton callus culture in dark conditions (*e.g.* Action, page 4, bottom). Further, although the Action asserts that embryogenic callus was cultured in media under complete darkness, referring to the cited reference, at page 169, right column, last paragraph, this paragraph does not describe such culture conditions for embryogenic callus. Instead, the callus referred to as grown in darkness at page 169 is non-embryogenic callus, growth of which having just been initiated and is being maintained, in the absence of embryogenesis. This is clearly the case because (1) the callus of the second and third sentences of this paragraph is not referred to as embryogenic callus; and (2) the following (fourth) sentence bridging pages 169-170 explicitly relates to embryogenic callus formation, further indicating the distinction between (non-embryogenic) callus and embryogenic callus. Applicants also note that Firoozabady (1993) teaches away from using darkness for embryogenic callus formation and growth, instead teaching that "high temperature and low light ($9\mu E/ m^{-2} s^{-1}$) were preferred..." [page 170, left column; underlining added].

In view of the many possible variables in such plant cell culture experiments and also the limitations of claims 39-41, 43, and 44, one of skill in the art of plant cell culture would have had no motivation to combine the teachings of Firoozabady (1993) and Rangan. Such a combination

would only be obvious with hindsight. Even if the references were combined, in total they offer no expectation of success, and as noted, teach away from use of limitations recited in these claims. In view of the above, the cited references therefore neither teach nor suggest all elements of the claims to one of skill in the art, and a *prima facie* case of obviousness has not been established. Applicants respectfully request that the rejection of claims 39-41,43, and 44 be withdrawn.

I. Rejection of Claims 50-52 and 54 under 35 U.S.C. § 103(a).

The Action rejects claims 50-52 and 54 under 35 U.S.C. § 103(a) as being unpatentable over Firoozabady (1993) in view of Davis *et al.*, Chi *et al.*, Firoozabady (1987), Rangan, and further in view of Gould. The rejection apparently relates to use of a sealing material. Applicants respectfully traverse.

The Action asserts that Gould teaches sealing of culture plates with PARAFILM (page 13, left column, last paragraph). As noted above, in *e.g.* section E, Gould relates to culture of shoot apical meristems, and would not be applied by one of skill in the art regarding embryogenic cotton cell culture. Further, for instance, the Specification (*e.g.* Example 7, and Table 9) teaches use of dark growth conditions in conjunction with PARAFILM sealing material. Gould explicitly describes culture under continuous high light conditions (90 $\mu E\ m^{-2}\ s^{-1}$; Gould, page 13, left column). Likewise, the present claims explicitly recite dark lighting conditions, limited light conditions, or under green light. Thus, Gould teaches away from use of the recited lighting conditions.

Use of the teachings of Gould are further asserted by the Action to be motivated by the advantage of reducing contamination. Applicants do not find any such motivation in the Gould reference. The only discussion regarding contamination relates to sterilization prior to isolation of explants (shoot apical meristems) at page 13, left column, "Culture Procedures". Such

contamination would be inherently present, if at all, in Gould's explants, and wrapping in PARAFILM would not alleviate such contamination. Thus no motivation to combine Gould with the other references is provided, and a *prima facie* case of obviousness has not been established by the Action.

Even if such motivation did exist for the sake of argument, given the numerous differences between the work of Gould and the other cited references, as well as the numerous defects regarding combining the other cited references, Applicants submit that it would not be clear to a skilled practitioner which of the numerous contradictory teachings among these 6 references to pick and choose to arrive at the presently claimed invention. The cited references therefore neither teach nor suggest all elements of the claims to one of skill in the art. The references in total also do not give a skilled practitioner any expectation of success, and teach away from the claimed method in instances outlined above. Applicants respectfully request that the rejection be withdrawn.

J. Conclusion

In view of the above, it is submitted that all of the rejections to the claims have been overcome, and the case is in condition for allowance.

The Examiner is invited to contact the undersigned at (214) 259-0931 with any questions, comments, or suggestions relating to the references patent application.

Respectfully submitted,

/Robert E. Hanson/

Robert E. Hanson
Reg. No. 42,628
Attorney for Applicants

SONNENSCHEIN NATH &
ROSENTHAL L.L.P.
1717 Main Street, Suite 3400
Dallas, Texas 75201
(214) 259-0911

Date: October 11, 2007